SELECTION AND CHARACTERIZATION OF CHINESE HAMSTER OVARY CELLS RESISTANT TO THE CYTOTOXICITY OF LECTINS

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SUMMARY

Chinese hamster ovary (CHO) cells selected in a single step for resistance to the cytotoxicity of the lectin from red kidney beans (PHA) behave as authentic somatic cell mutants. The PHA-resistant (PhaR) phenotype is stable in the absence of selection; its frequency in a sensitive population is increased several-fold by mutagenesis; and it behaves recessively in somatic cell hybrids. The activity of a specific glycosyl transferase which transfers N-acetylglucosamine (GlcNAc) to terminal α-mannose residues is dramatically reduced (~<5% of the activity detected in wild-type CHO cells) in several independent PhaR clones. These clones also exhibit (a) a decreased ability to bind [125I]-PHA; (b) a marked resistance to the cytotoxicity of wheat germ agglutinin (WGA), Ricin (RIC) and Lens culinaris agglutinin (LCA); (c) a 4- to 5-fold increased sensitivity to the cytotoxicity of concanavalin A (Con A); (d) an increased ability to bind 125I-Con A; and (e) decreased surface galactose residues—all properties consistent with the specific loss of the GlcNAc transferase activity. The lectins WGA, RIC, LCA and Con A have also been used to select, in a single step, resistant clones from each of two complementary CHO auxotrophic lines. These lectin-resistant clones have been characterized by their ability to survive cytotoxic doses of PHA, Con A, WGA, RIC or LCA, and 4–5 “lectin-resistance” phenotypes have been demonstrated. Complementation data is being sought by somatic cell hybridization. Preliminary results show that two phenotypically-distinct Con A8 mutants are complementary in that hybrid cells formed between them exhibit wild-type sensitivity to Con A.

Key words: somatic cell genetics; lectins; membrane mutants; glycosyl transferases; complementation.

There has been increasing evidence in recent years that cell membranes play a multiplicity of complex roles in cell function. Among the several approaches that can be made to the elucidation of these roles, genetic analysis offers some particular advantages. It can be expected that a variety of structural and functional properties of membranes will be altered by mutation. At the same time each particular genetic event will lead to a specific modification which can then be studied comparatively in isogenic cell lines. The strength and precision of the genetic approach to studies of over-all cell function and regulation, including that of cell membranes, has already been demonstrated clearly with prokaryotes. For these reasons, our laboratory initiated a program a few years ago designed to exploit genetic methods for the study of membrane structure and function. As might be expected, mutants involving a variety of membrane alterations have already been found. Ouabain-resistant mutants are altered in the Na/K activated ATPase situated in the membrane (1), and colchicine-resistant mutants are altered in their permeability to colchicine and a variety of other drugs (2).

In our own studies, we have been interested in membrane alterations engendered by mutation to resistance to the cytotoxicity of plant lectins. In addition to their known properties as mitogens and agglutinins, lectins are also toxic.
to some types of cells cultivated in vitro (3, 4). There is evidence that lectins exert their mitogenic and agglutinating action by binding to exposed carbohydrate residues of the cell membrane. Although in many cases the mechanism of cytotoxicity is unknown, binding at the cell membrane is probably a necessary first step in this process. Therefore it seemed reasonable to expect that, of mutants selected for their resistance to lectin cytotoxicity, some would show decreased binding of the lectin, which might reflect important modifications in the structure and/or conformation of the membrane.

The cell line we have chosen for our studies is the Chinese hamster ovary line (CHO) originally isolated by Puck and co-workers (5). This line has several advantages for genetic study: ease of manipulation; ability to grow in suspension culture; a relatively stable karyotype; a high plating efficiency; and the existence of several auxotrophic CHO lines (6). The feasibility of selecting lectin-resistant CHO lines was originally demonstrated by Wright (7), who isolated and studied concanavalin A-resistant cells.

In our laboratory, we have studied cells resistant to the phytohemagglutinin from *Phaseolus vulgaris* (PHA), and subsequently a variety of other lectin-resistant (Le-) cells. Our approach has been to isolate Le- cell lines, and then to examine their genetic, physiologic and biochemical properties. We have chosen two auxotrophs as parental lines—one which requires proline for growth (Pro-) and a second one which requires glycine, adenosine and thymidine but is reverted for the proline requirement (Gat-Pro+). Since a great deal of the detailed work describing these studies has either been published (8, 9), or is in press (10), or in preparation, in this paper we present mainly an overview, including only experimental data which is not to appear elsewhere.

**Isolation of PHA-resistant (Pha*) cells.** In the presence of 10–25 μg/ml PHA and the survivors were scored. Most of the colonies that arose were picked and shown to retain their PHA-resistance after subsequent culturing in nonselective media. Different CHO clones were plated in the presence of 10–25 μg/ml PHA and the survivors were scored. Most of the colonies that arose were picked and shown to retain their PHA-resistance after subsequent culturing in nonselective media.

with or without mutagenesis, and at least 98% of them remain resistant on subsequent culture in the absence of the selecting agent. In one study, cells retained their Pha* phenotype after being continuously cultured for 300 generations in the absence of PHA (8). The karyotype of Pha* cells is essentially identical to that of the parental line (8).

**Genetic characterization of Pha* cells.** As indicated above, Pha* cells breed true. Further genetic characterization of the system has been made by measuring the rate of mutation to PHA-resistance using the Luria-Delbruck Fluctuation Test (11), by examining the effects of a mutagen, and by determining the behaviour of the marker in somatic cell hybrids.

The results of the Luria-Delbruck Fluctuation Test are shown in Table 2. Two conclusions can be derived from this data. First, the rate of generation of Pha* cells is nonrandom and is therefore compatible with a mutation event. Secondly, the observed rate of mutation, using the Median method of calculation (12), is 1.5 ± 0.3 × 10⁻⁶ per cell per generation. The frequency with which Pha* cells can be isolated in hybrids formed between the two parental auxotrophs is of the order of 1 in 2 to 4 × 10⁶ viable cells. This is to be compared with the frequency in quasi-diploid cells of 1 to 2 in 10⁸.